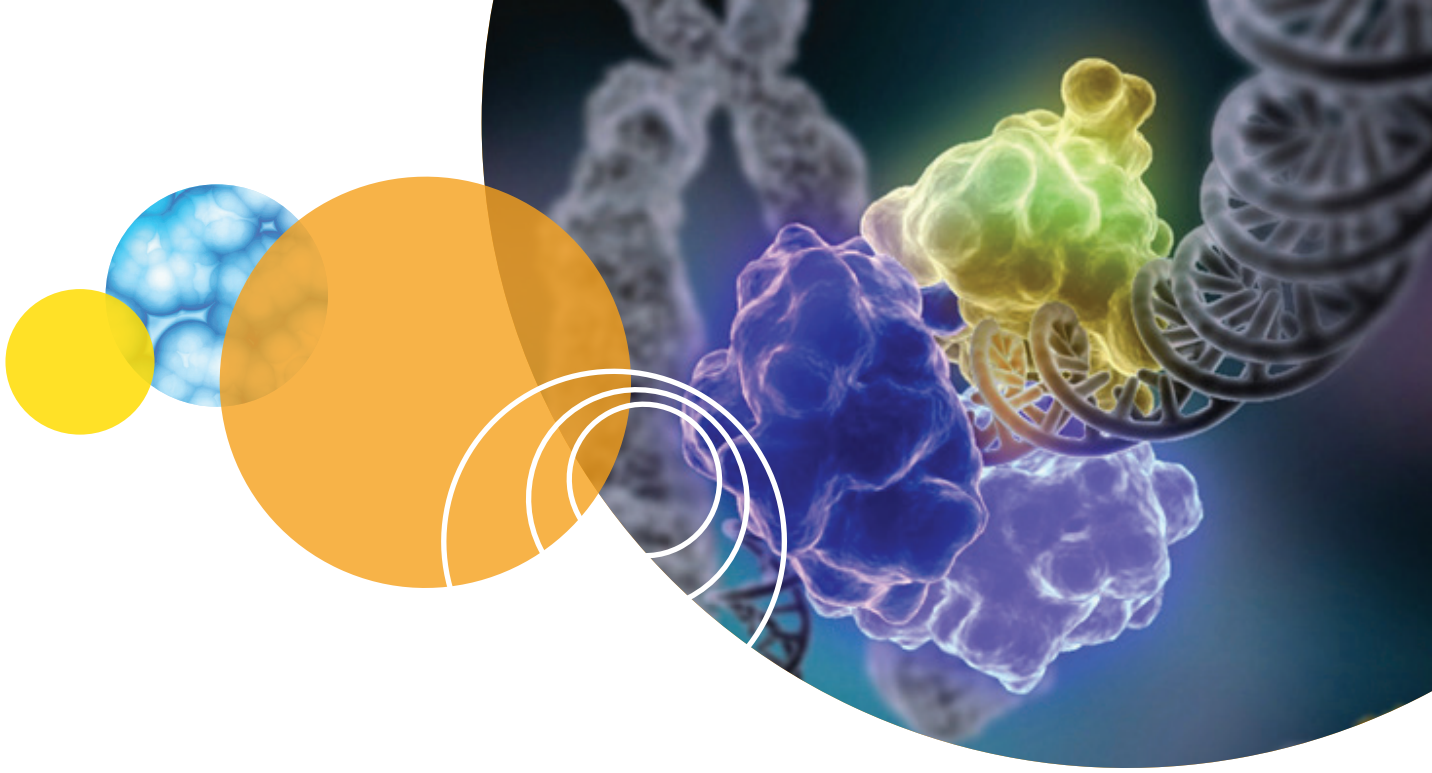

Detect Nucleic Acids and Proteins Like a Pro

Accurate and sensitive detection of nucleic acids and proteins are critical to many experiments. Molecular Devices SpectraMax® microplate readers support a multitude of assays for nucleic acids and protein detection. Combined with the analysis capabilities of SoftMax® Pro Software, we provide quantitation in an easy-to-read, user-customizable report format.

eBook contents

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Quant-iT PicoGreen dsDNA assay with SpectraMax microplate readers

Double-stranded DNA is typically quantitated in microplate readers by measuring the absorbance of the DNA solution at 260 nm. However, this method is only able to measure down to about 250 ng/mL on a typical absorbance microplate reader. For biological applications involving small samples, such as purification of DNA fragments for subcloning and quantitation of DNA amplification products, more sensitive methods are needed. The Quant-iT PicoGreen dsDNA Assay from Molecular Probes is more specific for DNA and is about 1000 times more sensitive than traditional absorbance methods. The dynamic range of this assay in microplate format is from 250 pg/mL to 1000 ng/mL with a single dye concentration.

This application note demonstrates that with SpectraMax® fluorescence microplate readers and the Quant-iT PicoGreen assay, users can reliably measure down to at least 100 pg/mL of double-stranded DNA.

- Sensitive fluorescent quantitation of DNA down to 63 pg/mL
- Linear dynamic range spanning four orders of magnitude
- Easy analysis of results with preconfigured protocol in SoftMax Pro Software

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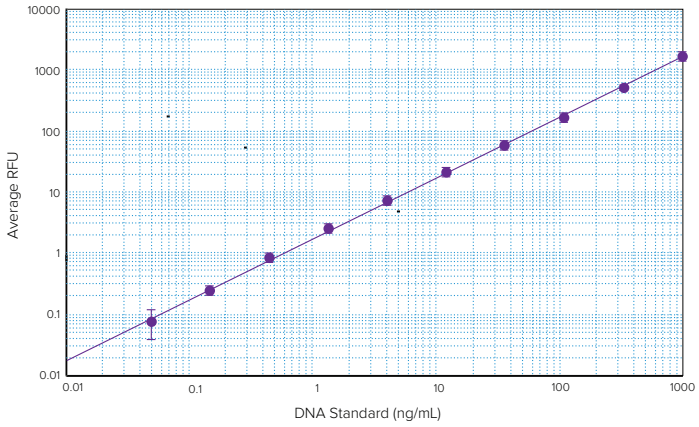
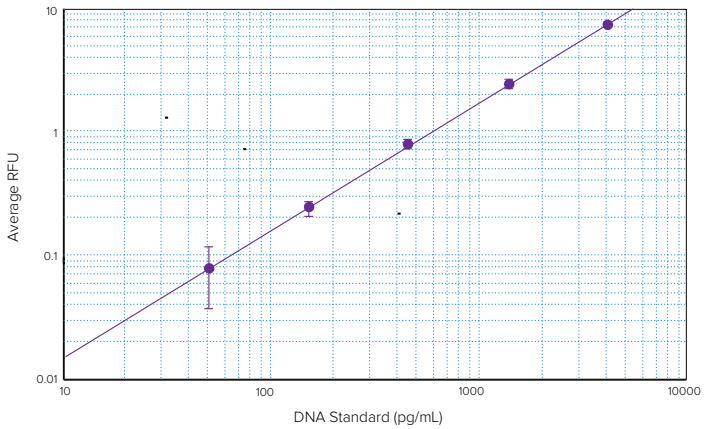


DNA standard curve. DNA standards ranging from 1000 ng/mL to 50 pg/mL were assayed using the Quant-iT PicoGreen dsDNA Assay and SpectraMax M5 microplate reader. The standard curve was plotted using a log-log curve fit in the SoftMax® Pro Software (r^2 = 1.000).



Lower end of DNA standard curve. Plot of the lower end of the DNA standard curve, ranging from 4.1 ng/mL to 50 pg/mL (r^2 = 1.000).

DNA and RNA absorbance measurements using SpectraMax microplate readers

Ultraviolet (UV) measurements in microplates became possible when Molecular Devices introduced the first UV-capable microplate reader. Since then, microplate measurements of DNA, RNA, and proteins have become very popular.

However, microplate assays require awareness of the optical properties of the microplate materials and more attention to technique than do traditional cuvettes in order to get accurate absorbance results, especially in the UV range. Lack of attention to these details is the most frequent cause of difficulty in adapting assays to microplates. Absorbance measurements made through microplates are subject to pathlength variability and are vulnerable to interference from surface effects at the air/liquid interface. Modern microplate readers, with smaller optical beams than older wide-beam plate readers, are much more vulnerable to spurious readings due to dust. Particles in the light beam at the time of a read can cause artifactual absorbance spikes of up to 0.3 OD. Therefore it is especially important that sample solutions be free of particles. All of the above factors must be kept in mind in order to get accurate and reproducible absorbance results in microplates.

Here, we provide guidelines for optimizing DNA/RNA absorbance measurements in SpectraMax® microplate readers.

- Direct quantitation of nucleic acids without standard curves
- DNA quantitation down to 250 ng/mL
- Preconfigured protocol with SoftMax Pro Software

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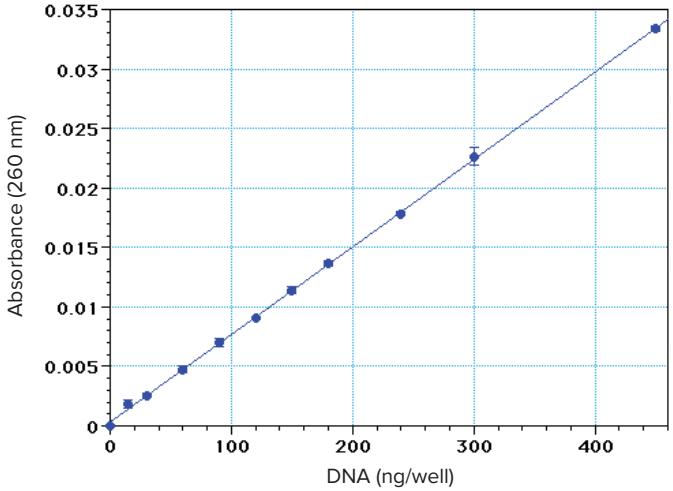
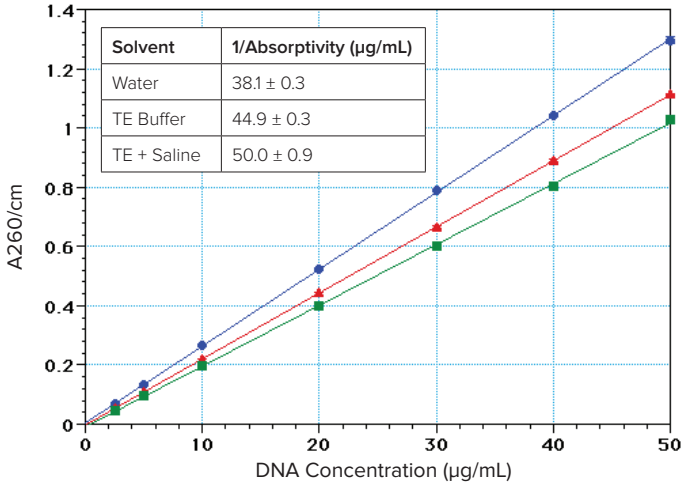


Standard DNA Curve. Standard curve obtained with calf thymus DNA dissolved in water. Triplicate 300 µL aliquots were placed in a Costar UV microplate and read at 260 nm with PathCheck applied.



Solvent	1/Absorptivity (µg/mL)
Water	38.1 ± 0.3
TE Buffer	44.9 ± 0.3
TE + Saline	50.0 ± 0.9

DNA/RNA estimation using absorptivity. Effect of ionic strength on DNA absorption at 260 nm. The DNA (Sigma Type I "Highly polymerized", Cat. No. 1501) was dissolved in deionized water or TE buffer (10 mM Tris, 1 mM EDTA, pH 7.4) or TES (TE buffer + 0.9% NaCl). Insert: 1/absorptivity values; Average of 7 concentrations (2.5–50 µg/mL), 4 replicates each.

Measuring cell proliferation using the CyQUANT Cell Proliferation Assay

Quantitation of cell proliferation using fluorescence allows one to easily monitor the effects of drugs and other experimental treatments on cell growth. The CyQUANT Cell Proliferation Assay Kit from Life Technologies is a sensitive, rapid and convenient way to quantitate cell growth using a fluorescence microplate reader. CyQUANT GR dye binds to cellular nucleic acids, allowing cell numbers to be calculated from a standard curve. Because DNA-to-RNA ratios can vary over the course of the cell cycle, the CyQUANT kit allows users to determine cell numbers using RNase-digested cell lysates and a nucleic acid standard curve.

Here we describe how to use the CyQUANT kit with SpectraMax microplate readers and SoftMax® Pro Software. Two methods are detailed. In the first, cellular proliferation is quantitated using a cell-based standard curve. In the second, cellular proliferation is quantitated using RNase-treated cell samples and a DNA standard curve.

- Rapid and convenient quantitation of cell growth
- Sensitive detection of as few as 50 cells
- Easy data analysis with preconfigured SoftMax Pro Software protocol

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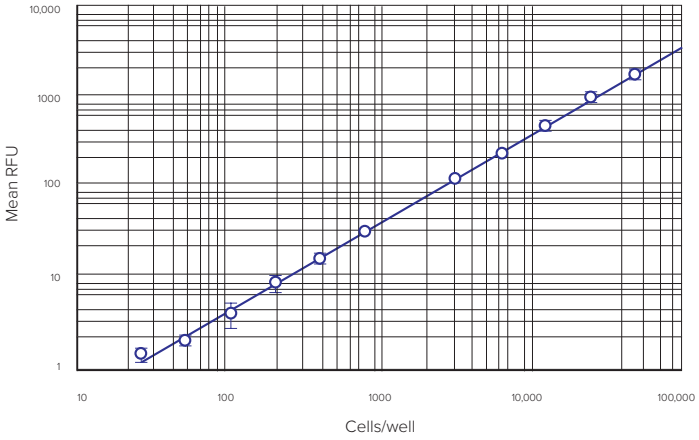
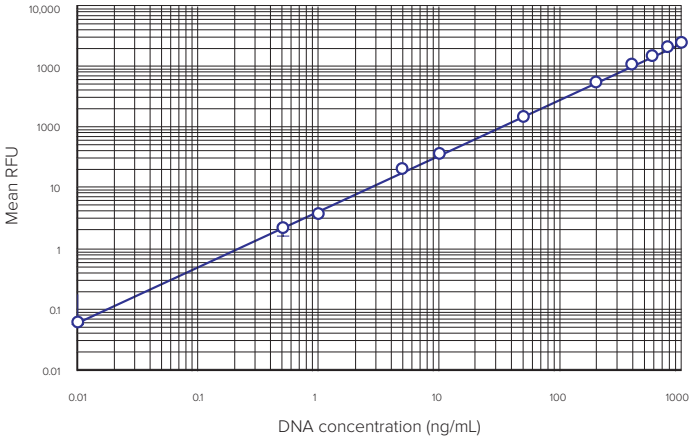
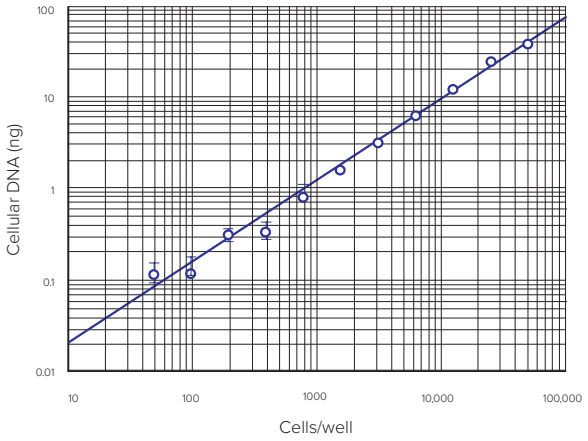


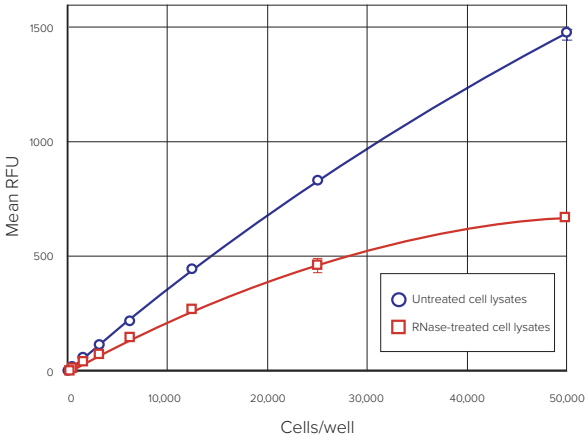
Cell-based standard curve. Cell densities (from 25 to 50,000 cells per well) were assayed.



DNA standard curve. DNA standard curve obtained using the CyQUANT assay kit.



Cellular DNA vs. cells-per-well. Cellular DNA concentration vs. number of RNase-treated cells per well.



Comparison between RNase-treated and untreated cells. RFU vs. Cells/well for RNasetreated cells compared to untreated cells. Blue circles, untreated cell lysates; red squares, RNasetreated cell lysates.

Optical density measurements automatically corrected to a 1-cm pathlength

UV/VIS spectrophotometers and microplate readers differ fundamentally in their beam geometry. In spectrophotometers, samples are read through cuvettes or tubes with a horizontal (cross-sectional) light path. The horizontal light beam and the customary 1 cm pathlength make assays based on extinction coefficients straightforward and allow easy comparison of results between labs. In microplate readers, the vertical light beam results in a pathlength that depends on the volume of fluid in each well.

The variable pathlength in microplates has made it difficult to perform extinction-based assays and confusing to compare results obtained in a microplate reader with those obtained in a spectrophotometer. This problem has been remedied by the introduction of the SpectraMax® microplate reader and its PathCheck® feature, the ability to determine the pathlength in each well of a microplate and automatically normalize the absorbance value to a 1-cm pathlength. This application note discusses the principles on which PathCheck is based, and gives specific instructions for using it with a SpectraMax microplate reader and Softmax® Pro Software.

- Automatic correction for variable microplate well volumes for more accurate results
- Absorbance-based quantitation without standard curves
- Temperature-independent pathlength correction

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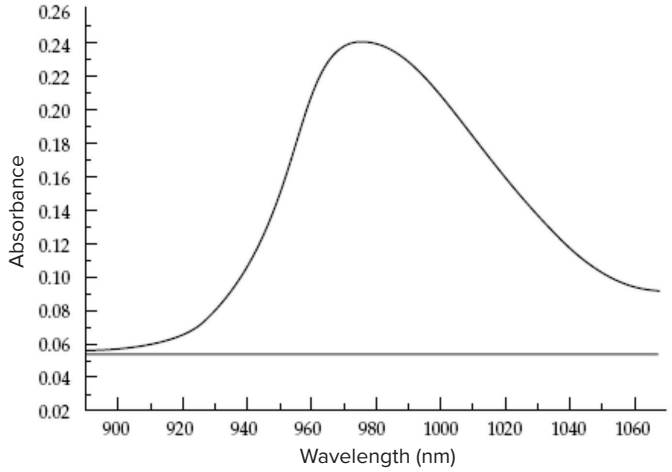
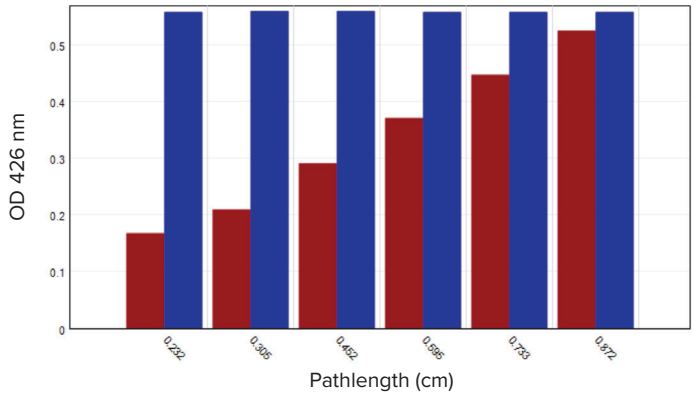


Absorbance spectrum of water. The absorbance spectrum of water is shown with absorbance peak at 977 nm.



OD_{426} without and with pathlength correction. Red bars, OD values without pathlength correction; blue bars, pathlength-corrected OD values. Average pathlength for samples ranging from 75 to 300 µL are displayed on the y axis

Protein quantitation with NanoOrange assay

Traditional photometer methods, such as absorbance 280, BCA, Bradford or Lowry assays, are not very sensitive in microplate format. The dynamic range of NanoOrange® Protein Quantitation Kit from Life Technologies in microplate format is 10 ng/mL to 10 µg/mL. The data presented in this application note confirms the dynamic range and lower detection limit.

The NanoOrange assay, when run on a Gemini XPS Microplate Reader or other SpectraMax fluorescence microplate reader with SoftMax® Pro Software, is a quick, sensitive detection method for proteins. The analysis capabilities of SoftMax Pro Software provide quantitation in an easy-to-read, user-customizable report format.

- Greatly improved sensitivity over absorbance methods
- Wide dynamic range from 10 ng/mL to 10 µg/mL
- Easy data acquisition and analysis with preconfigured SoftMax Pro Software protocol

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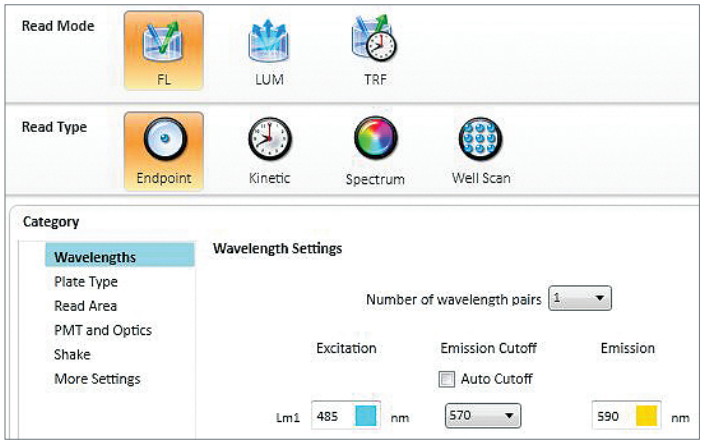
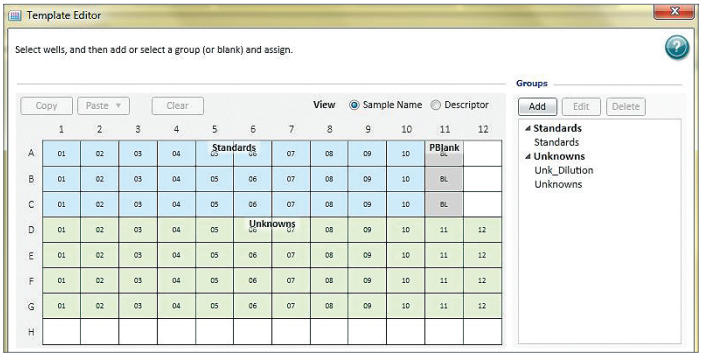
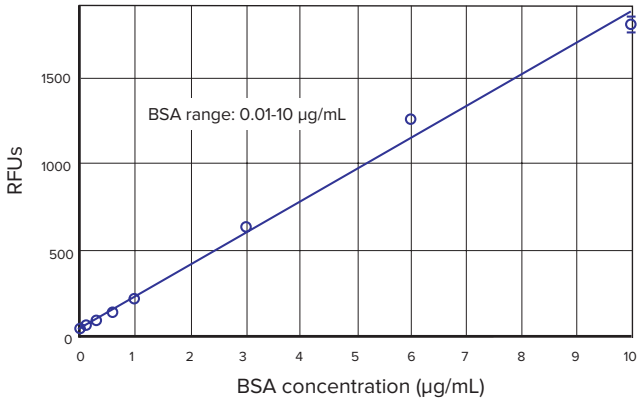


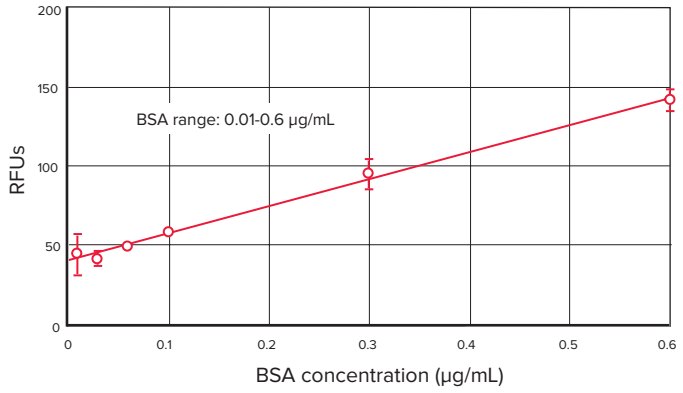
Plate reader settings for NanoOrange assay. Typical settings for SpectraMax readers with fluorescence detection mode are shown.



Template setup. Assigning wells to 'Standards' and 'Unknowns' groups in the Template Editor enables automatic plotting of the standard curve as well as data analysis.



NanoOrange standard curve. Standard curve obtained using BSA standards and a linear curve fit.



Standard curve low end detail. Detail of the lower end of the standard curve shown in figure to the left.

Protein quantitation with Bradford and ELISA assays

Endpoint readers are prolific in the laboratory since absorbance has become the detection of choice for many applications. Examples include ELISAs for quantitation of cytokines and protein concentration determination using the Bradford assay. Here we compare the performance of the Molecular Devices EMax® Plus Microplate Reader to the VMax reader using a Bradford protein quantitation assay. In addition, a performance comparison is made between the new EMax Plus Microplate Reader and the EMax Endpoint Reader using a sandwich ELISA for the quantitation of mouse/rat IL-22.

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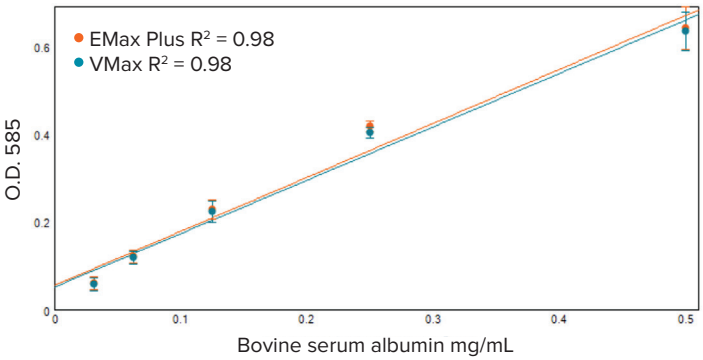
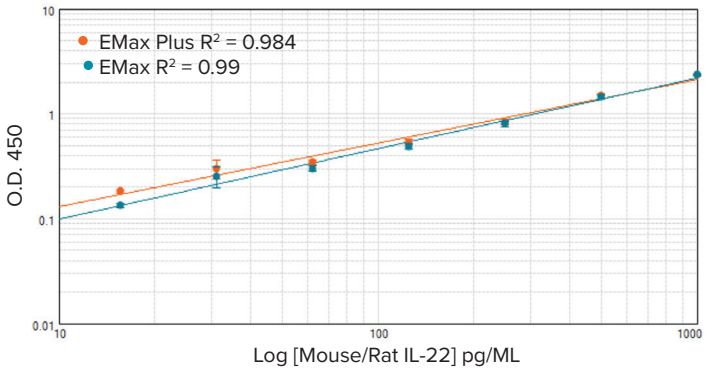
- 8 filters come standard to cover a wide range of applications
- Compact footprint
- Walk-up usability
- Preconfigured protocols with SoftMax Pro Software



Absorbance reader comparison with Bradford protein assay. The Bio-Rad Bradford Protein Assay was used to determine the concentrations of bovine serum albumin in a standard curve. Signal comparison from both instruments was nearly identical. The assay maintained linearity across five dilutions with R^2 values = 0.98.



Absorbance reader comparison with ELISA assay. A Mouse/Rat IL-22 Quantikine ELISA from R&D Systems was used to compare performance of the EMax Plus and the EMax endpoint readers in an absorbance assay. An IL-22 standard curve was prepared and a sandwich ELISA performed using the MultiWash+ Plate Washer in strip mode to wash the wells. After reading the ELISA plate on both readers, each standard curve was nearly identical.

Fluorescence-based quantitation of small DNA samples

Measurement of DNA using UV absorbance is a common method allowing quantitation of many sample types. However, some applications require quantitation of very low sample volumes or concentrations due to preparation method or scarcity of source material. For these applications, fluorescent DNA quantitation methods offer greater sensitivity and the ability to use smaller sample volumes with lower concentrations.

The SpectraDrop™ Micro-Volume Microplate for SpectraMax® microplate readers offers higher throughput than any other low-volume microplate, with up to 64 samples per plate. Samples with volumes as low as 2 µL can be measured. The SpectraDrop microplate incorporates a specially designed adapter and a slide pair whose optical clarity allows measurements in absorbance and fluorescence modes to meet your application needs.

- DNA detection down to 2.5 pg/µL
- Unmatched throughput
- Calibration-free setup
- Easy-to-clean design

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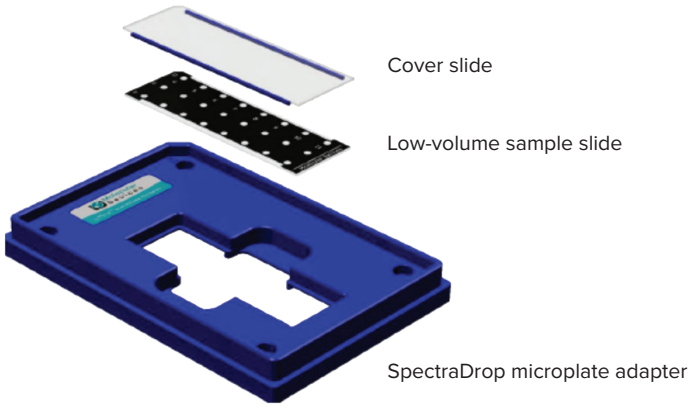
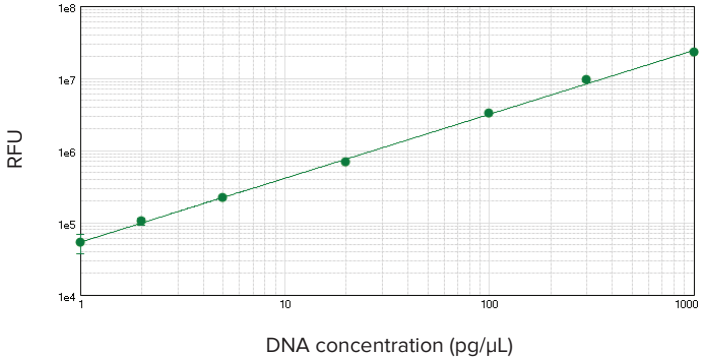


SpectraDrop microplate configuration. The low-volume sample slide has a mask delineating spots or 'wells' that hold 24 or 64 samples. The cover slide has 0.5-mm or 1.0-mm spacers for use with 2-µL or 4-µL samples, respectively.



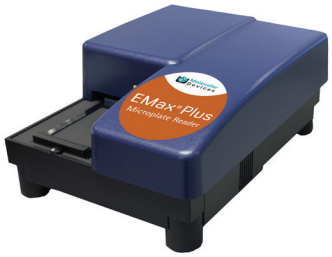
Standard dsDNA curve. dsDNA standard curve using SpectraDrop Micro-Volume Microplate on the SpectraMax Paradigm reader. The lower limit of detection for dsDNA was 5 pg/well, or 2.5 pg/µL. Standard curve $r^2 = 0.999$. Similar results were obtained with the SpectraMax M5e reader.

Systems for detection of nucleic acids and proteins

For detailed information, select the images or text.

SpectraMax® i3x Multi-Mode Microplate Reader

Gemini XPS™ Microplate Reader



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SpectraDrop™ Micro-Volume Microplate

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